Inputs from wastewater treatment plant effluent influence the temporal variability of nutrient uptake in an intermittent stream

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Abstract

Effluents from wastewater treatment plants (WWTP) affect water chemistry and in-stream nutrient uptake capacity from receiving freshwaters, thus altering the amount and fate of nutrients exported. In Mediterranean regions, the dilution capacity of receiving streams to buffer the WWTP biogeochemical fingerprint can vary seasonally due to changes in hydrologic conditions. We assessed the temporal patterns and controls on nutrient uptake in an intermittent Mediterranean stream receiving WWTP effluent inputs. We compiled data on longitudinal profiles of ambient concentrations of dissolved inorganic nitrogen and phosphorus along a 800 m reach on 47 sampling dates between 2001 and 2017 that cover a wide range of hydrological conditions. Data were used to estimate net nutrient uptake in the receiving stream. Ammonium concentration decreased along the reach in 72% of dates, and these decreases were coupled with increases of either nitrite or nitrate. This phenomenon suggests that the stream acted as a hot spot of nitrification. Conversely, concentration of phosphorus did not show any longitudinal pattern in 75% of dates, suggesting that uptake and release processes for this element were commonly counterbalanced. Finally, ammonium net uptake decreased when the stream had a low dilution capacity, suggesting that excess of available nutrients associated with WWTP inputs control temporal variation of the bioreactive capacity of the receiving streams. Overall, this study suggests that water management should consider the biogeochemical interplay between WWTP operation and the functioning of receiving streams as a strategy to improve stream water quality in urban landscapes.

Introduction

Effluents from urban wastewater treatment plants (WWTP) are point sources of nutrients, organic matter, emergent pollutants, and microbes, some of which are pathogens, to receiving streams (Marti et al. 2004; Meng et al. 2013; Mußmann et al. 2013; Kay et al. 2017; Pascual-Benito et al. 2020). These point source inputs generate abrupt physical and chemical discontinuities along the river continuum that deteriorate water quality (Brion and Billen 2000; Gray 2004; Jin et al. 2007, Martí et al. 2010). In addition, the excessive enrichment of nutrients and organic matter can enhance the growth of primary producers and the activity of heterotrophic microbial assemblages associated with the breakdown of organic matter (Gücker et al. 2006; Wakelin et al. 2008), thus modifying the composition, functioning and trophic state of the microbial communities in receiving streams (Miltner and Rankin 1998; Jin et al. 2007; Beyene et al. 2009; Aristi et al. 2015).

Freshwater ecosystems have an important capacity to store, transform and retain nutrients during downstream transport because in-stream biota relies on nutrients to develop (Peterson et al. 2001; Mulholland et al. 2008). In-stream biota takes up nutrients from the water column via different assimilatory and dissimilatory biogeochemical uptake pathways (Peterson et al. 2001; Bernot and Dodds 2005; Ribot et al. 2017). Assimilatory uptake refers to the transitory uptake of inorganic nitrogen (N) and phosphorus (P) into biomass during biosynthetic processes (Tank et al. 2018). Furthermore, P uptake can also be controlled by sediment adsorption and geochemical processes (Trentman et al. 2020); and N can also undergo different energy-yielding dissimilatory pathways (Kuypers et al. 2018). The most relevant are nitrification, the aerobic oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) (Prosser 1989; Lin et al. 2009), and denitrification, the reduction of NO_3^- to N_2 gas under sub-oxic conditions during organic matter mineralization (Seitzinger et al. 2006). Together, this in-stream bioreactive capacity may contribute to alleviate the excess of nutrients from WWTP in receiving stream. Previous studies have shown that WWTP effluent inputs diminish the efficiency of receiving streams to take up N and P because excess nutrient loads can cause the saturation of nutrient demand by in-stream biota (Martí et al. 2004; Grimm et al. 2005; Gücker et al. 2006; Merseburger et al. 2011). Yet, longitudinal decreases in N and P ambient concentration have been observed in WWTP receiving streams, suggesting that even when the nutrient retention efficiency is low, the stream biota can reduce to some extent the pervasive concentrations of nutrients in those systems (Haggard et al. 2005; Ribot et al. 2012; Bernal et al. 2020). In this regard, previous studies show that stream reaches located downstream from WWTP can become hot spots of nitrification because of the colonization of the streambed biofilm by ammonium oxidizing bacteria from the WWTP active sludge together with the high NH_4^+ concentration (Mußmann et al. 2013; Merbt et al. 2015).

The influence of WWTP inputs on nutrient uptake in receiving streams can vary seasonally because most processes involved on in-stream nutrient cycling strongly rely, among other factors, on the hydrological conditions and nutrient availability (Peterson et al. 2001; Newbold et al. 2006). During high flows, the efficiency for stream biota to take up nutrients is low because the interaction between the active streambed and the water column height and the water residence time are also low and therefore, downstream transport might overwhelm in-stream biogeochemical processing (Martí et al. 1997; Algerich et al. 2008). Conversely, during low flows, receiving streams can act as net sinks of nutrients because higher water residence time favors the interaction between in-stream biota and bioreactive solutes (Ribot et al. 2012; Rahm et al. 2016; Bernal et al. 2020). Besides influencing water residence time, stream hydrology also affects water chemistry, especially in reaches located downstream of WWTP inputs. For instance, during low flows, the dilution capacity of the receiving stream decreases and water chemistry is basically driven by the WWTP inputs; and hence, nutrient concentrations in the receiving streams are high (Keller et al. 2014; Bernal et al. 2020). Previous studies have shown that nutrient uptake demand is inversely related to nutrient concentration (Earl et al. 2006; Newbold et al. 2006). Therefore, it is also expected that, during extremely low flows, the capacity of stream biota to take up nutrients can be overwhelmed by the high nutrient concentrations. Nevertheless, the extent to which hydrology will interact with chemistry to control temporal variation of nutrient uptake in receiving stream is not fully understood yet. In urban landscapes with water scarcity, such as the Mediterranean region, the temporal patterns of hydrology

and dilution capacity in receiving streams as well as the biogeochemical derived effects could be magnified because the dilution capacity of the receiving stream can largely vary on an annual basis from 0% to >95% (Martí et al. 2010; Bicknell et al. 2020).

Most existing studies on nutrient uptake in WWTP-receiving streams have focused on quantifying the effects of the WWTP inputs (Gücker et al. 2006; Merseburger et al. 2011; Arnon et al. 2015), but less information is available on assessing its temporal variation and associated controlling factors (Bernal et al. 2020). However, understanding how in-stream nutrient processing varies over time in rivers that receive inputs from WWTP is essential for an integrated management of water resources and their quality in urban landscapes, especially under water scarcity conditions. In this context, we aimed to assess the temporal variability in nutrient uptake and factors controlling it in a Mediterranean stream with an intermittent hydrologic regime that receives the inputs from a WWTP effluent. To this aim, we compiled data on longitudinal profiles of ambient nutrient concentrations collected along a 800 m reach downstream of a WWTP input on different dates ($n = 47$) between August 2001 and October 2017 that encompassed the four seasons of the Mediterranean climate and covered a wide range of hydrological conditions. From this dataset, we calculated in-stream net nutrient uptake and characterized each sampling date in terms of hydrology and water chemistry to assess the relative influence of these factors on the temporal variation of net nutrient uptake.

Material And Methods

Study site

This study was conducted in the catchment of La Tordera river, which is located near Barcelona (NE of Catalonia, Spain). Within this catchment, we selected a third-order stream reach immediately located downstream of the input from the WWTP effluent of the municipalities of Sant Esteve and Santa Maria de Palautordera (lat. $41^{\circ}41'3.47''\text{N}$, long. $2^{\circ}27'33.19''\text{W}$). The reach is 800 m long with no major lateral inputs (e.g., tributaries). Channel morphology is characterized by a low sinuosity, a run–riffle sequence with a few shallow pools, and a slope close to 1%. Streambed substrata is dominated by cobbles (34%), pebbles (22%), and boulders (22%) (Merseburger et al. 2005). The reach is flanked by a dense canopy of riparian trees, with some areas of sparse vegetation (Bernal et al. 2020). At the study location, the mean annual stream discharge is 267 ± 115 L/s, but it varies several orders of magnitude within and among hydrological years due to the intrinsic characteristics of Mediterranean climate of the region (Merseburger et al. 2005). In summer, the stream commonly dries out upstream of the WWTP input.

The WWTP treats 17,900 population equivalents, where 1 population equivalent is the biodegradable organic-matter load corresponding to a biological O_2 demand (BOD5) of 60 g O_2/d . This WWTP lacks tertiary treatment; and, despite it fulfils de legislation requirements concerning urban waste-water treatment for the quality of the effluent (Council Directive 91/271/EEC), it represents a high contribution of dissolved inorganic N (DIN) and soluble reactive P (SRP) to the receiving stream (Bernal et al. 2020). Nutrient concentrations in the WWTP effluent vary over seasons, mainly because of changes in the biologic activity of the WWTP activated sludge (Bernal et al. 2020). Nevertheless, > 90% of the DIN in the WWTP effluent is in the form of NH_4^+ (Merseburger et al. 2005). The WWTP discharge is relatively constant over the year (~ 27.4 L/s); however, its contribution to stream discharge varies seasonally from 3–100% due to the stream hydrologic variability. The maximum contribution of the WWTP effluent to stream discharge commonly occurs during summer low flow.

Here, we compiled data on longitudinal profiles of concentrations of NH_4^+ , nitrite (NO_2^-), NO_3^- , and SRP collected along this reach during different studies ($n = 47$ sampling dates) encompassing an overall period from August 2001 until October 2017. We used this data set to estimate net nutrient uptake for the different DIN and P forms to examine its temporal variability in the receiving stream. Despite the sampling periodicity is irregular, the data set covers a wide range of hydrological conditions and it is representative of the four climatic seasons of this Mediterranean region (winter $n = 7$; spring $n = 16$; summer $n = 13$; and autumn $n = 11$).

Field sampling and laboratory analysis

We defined eight sampling sites along the reach, which were evenly distributed every 100 m. In addition, we selected a sampling site upstream of the WWTP that served as control to assess the relative contribution of the WWTP input to the receiving stream. On each sampling date, we took surface water samples from each sampling site (3 replicates) using 100 mL acid-washed plastic syringes. Water samples were immediately filtered through a GF/F glass fiber filters (Whatman ®) with a pore of 0.7 μm and kept refrigerated on ice in the field. Once in the laboratory, we stored samples at -20°C until subsequent analysis of NH_4^+ , NO_3^- , NO_2^- , and SRP. At each sampling site, we also recorded electrical conductivity (EC, in $\mu\text{S}/\text{cm}$), water temperature (in $^{\circ}\text{C}$), and dissolved oxygen concentration (DO, in mg/L) using WTW 340i portable sensors. Furthermore, on each sampling date, we estimated stream discharge (Q , in L/s) based on measurements of the wetted width (w , in m), the average of water velocity (v , in m/s) and water column depth (h , in m) of a representative cross-sectional transect (Gordon et al., 2004) located 200 m below the WWTP effluent.

Concentrations of NH_4^+ , NO_3^- , NO_2^- and SRP were analyzed following standard colorimetric methods (APHA, 1995) on a continuous flow autoanalyzer FUTURA (Alliance Instruments). Concentration of DIN for all the data set ($n = 46$) was estimated as the sum of NH_4^+ and NO_3^- . Concentration of NO_2^- was not included in DIN estimates because values were only available for half of the dates; and in average, it represented <

1% of DIN concentration. We also calculated the molar ratios between DIN and SRP and between NO_3^- : NH_4^+ concentrations as proxies of potential N or P limitation and relative proportion of oxidized and reduced DIN forms, respectively. Samples were analyzed at the Labqa Service of the Centre for Advanced Studies of Blanes (CEAB-CSIC).

Parameter calculations

On each sampling date, we estimated the contribution of the WWTP effluent to stream discharge (in %) by using conductivity as a conservative tracer as follows:

$$\text{Contribution WWTP (\%)} = 100 * (EC_1 - EC_{UP} / EC_{WWTP} - EC_{UP}) \text{ Eq. 1}$$

where EC_1 is the electrical conductivity at the first sampling site of the stream reach located at 100 m downstream of the WWTP. Empirical measurements in 2001 indicated that this distance from the WWTP input is enough to ensure a complete mixing of the effluent water with stream water under different hydrological conditions. EC_{UP} is the electrical conductivity at the sampling site located upstream of WWTP; and EC_{WWTP} is the electrical conductivity of the WWTP effluent. Then, we estimated the hydrologic dilution factor of the receiving stream (DF, in %) as the inverse of the WWTP contribution to discharge (i.e. $DF = 100 - \text{Contribution WWTP}$). Thus, low DF values indicate that the stream flow is low relative to WWTP inputs (Gupta 2008). Some studies have indicated deleterious effects from WWTP inputs on receiving streams at $DF < 40$ (Keller et al. 2014; Romero et al. 2019). Therefore, in engineering practice, this threshold can be used to determine risk vs no risk conditions in terms of the vulnerability of the stream to WWTP inputs.

We used data from the longitudinal profiles in nutrient concentrations along the reach to estimate net nutrient uptake following (von Schiller et al. 2011). Specifically, for each nutrient, we used the following first-order equation to estimate the net uptake coefficient per unit length of the reach (k , in $1/m$):

where C_1 is the nutrient concentration at the first sampling site of the reach below the WWTP effluent (in mgN/L or mgP/L); C_x is the nutrient concentration at sampling site located x meters from the WWTP effluent input; and EC_x is the electrical conductivity at that sampling site. We estimate k as the slope of the regression between the natural logarithm of nutrient concentration (corrected by the conductivity) and the distance from the WWTP effluent input of each sampling site. Longitudinal profiles of ambient nutrient concentrations represent the net balance between in-stream uptake and release processes; and thus, k values can be positive (uptake > release), negative (uptake < release), or ≈ 0 (uptake $\sim$ release) (Merseburger et al. 2005).

We used k to estimate the net uptake velocity (V_f , in mm/min), that describes the velocity at which a nutrient molecule is removed from the water column and is a proxy of in-stream nutrient demand (Bernhardt et al. 2002). Values of V_f will be positive, negative, or ≈ 0 depending on the value of k ; and are calculated as follows (Bernal et al. 2020):

Data analysis

We inferred potential seasonal differences in stream physical and chemical characteristics based on the variables measured on each sampling date (see Table 1) by using a non-parametric Kruskal-Wallis test (season as a factor) because data sets were not normally distributed and showed heteroscedasticity.

Table 1

Physical and chemical characteristics of the study stream, which receives inputs from a wastewater treatment plant effluent, measured on different seasons over the study period. For each variable, we report the range of values (minimum and maximum) and the number of data available (n) corresponding to all sampling dates. We also show the average $\pm$ Standard Error values of each variable considering data from each season separately. For each season, n indicates the number of sampling dates with available data. The results from Kruskal-Wallis non-parametric test to examine differences among seasons for each variable are shown; where n.s. indicates no significant differences (P-value > 0.05). Variables are: Temperature (T), discharge (Q), dilution factor of receiving stream (DF), electrical conductivity (EC), dissolved oxygen concentration (DO), concentrations of ammonium (NH₄⁺), nitrite (NO₂⁻), nitrate (NO₃⁻), dissolved inorganic nitrogen (DIN) and soluble phosphorus (SRP), and the molar ratios between nutrients (NO₃⁻:NH₄⁺ and DIN:SRP).

Parameters	All seasons			Winter		Spring		Summer		Autumn		P-value seasons
	n	Minimum	Maximum	n	Average $\pm$ SE	n	Average $\pm$ SE	n	Average $\pm$ SE	n	Average $\pm$ SE	
T (°C)	49	8	26	7	10 $\pm$ 0,2	16	16 $\pm$ 0,4	12	22 $\pm$ 0,5	11	15 $\pm$ 0,8	0,000
Q (L/s)	47	6	1639	7	351 $\pm$ 104	16	442 $\pm$ 59	13	55 $\pm$ 8	11	136 $\pm$ 28	0,001
DF (%)	47	0	100	7	56 $\pm$ 4	16	74 $\pm$ 4	13	34 $\pm$ 6	11	41 $\pm$ 6	0,015
EC (μ S/cm)	50	123	786	7	263 $\pm$ 18	16	249 $\pm$ 21	13	506 $\pm$ 34	11	429 $\pm$ 33	0,000
DO (mg/L)	28	3,4	11,6	5	10,8 $\pm$ 0,2	7	7,6 $\pm$ 0,7	6	6,1 $\pm$ 0,5	7	8,7 $\pm$ 0,5	0,026
NH ₄ ⁺ (mg N/L)	46	0,03	6,74	7	1,66 $\pm$ 0,34	16	1,38 $\pm$ 0,24	13	1,44 $\pm$ 0,19	10	1,49 $\pm$ 0,24	n.s
NO ₂ ⁻ (mg N/L)	24	0,004	0,024	4	0,01 $\pm$ 0,002	8	0,05 $\pm$ 0,020	5	0,13 $\pm$ 0,030	7	0,03 $\pm$ 0,006	n.s
NO ₃ ⁻ (mg N/L)	46	0,4	4,4	7	2,0 $\pm$ 0,1	16	1,2 $\pm$ 0,1	13	1,9 $\pm$ 0,2	10	2,2 $\pm$ 0,2	0,026
DIN (mg N/L)	46	0,9	8,3	7	3,7 $\pm$ 0,4	16	2,6 $\pm$ 0,3	13	3,4 $\pm$ 0,2	10	3,7 $\pm$ 0,3	n.s
SRP (mg P/L)	35	0,02	0,25	6	0,10 $\pm$ 0,02	10	0,18 $\pm$ 0,04	10	0,88 $\pm$ 0,14	9	0,54 $\pm$ 0,15	0,009
NO ₃ ⁻ :NH ₄ ⁺	46	0,04	28,96	7	1,35 $\pm$ 0,44	16	0,87 $\pm$ 0,2	13	3,12 $\pm$ 1,23	10	1,97 $\pm$ 0,76	n.s
DIN:SRP	35	1	157	6	35 $\pm$ 5	10	29 $\pm$ 4	10	6 $\pm$ 1	9	38 $\pm$ 9	0,009

We also examined how measured physical and chemicals variables contributed to the observed temporal variability among sampling dates by using a principal component analyses (PCA). The weight of a variable on a PCA component was considered significant when its loading was > 0.7. Unfortunately, some of the variables were not measured in all sampling dates. Therefore, to avoid a significant reduction of available sampling dates to be considered in the PCA analysis, we excluded from the analysis those variables with a high number of missing values (i.e., concentrations of DO, NO₂⁻, and SRP; and the DIN:SRP molar ratio; Table 1). In addition, we used non-parametric Kruskal-Wallis test on the scores of PC1 and PC2 to examine differences among seasons.

For each nutrient, we estimated the frequency of cases in which $V_f < 0$ (release > uptake), $V_f > 0$ (release < uptake) and $V_f \approx 0$ (release $\approx$ uptake) considering all data together to compare in-stream responses among nutrients, and separated by seasons to identify temporal patterns in dominant pathways of nutrient cycling. We also used a non-parametric Kruskal-Wallis test to test for seasonal differences on V_f for each particular nutrient. For this analysis, we only selected those dates when longitudinal profiles showed either a significant decline (i.e., uptake > release; V_f positive or net uptake) or increase (i.e., uptake < release; V_f negative or net release). In the case of V_f for DIN, we could not consider winter in the analysis because none of the longitudinal profiles of this season showed significant longitudinal trends.

Further, we used simple regression analysis between V_f for NH₄⁺ and V_f for NO₃⁻ or NO₂⁻ to examine potential relationships that may help inferring in-stream nitrification as a dissimilatory N uptake pathway in the study reach, as suggested in previous studies (Merseburger et al. 2005; Ribot et al. 2012; Bernal et al. 2020). Finally, to examine potential factors contributing to the temporal variability of net nutrient uptake of the receiving streams, we explored the relationship between V_f of the different nutrients and the scores from PC1 and PC2 used as variables integrating stream physical and chemical characteristics of the receiving stream on the different sampling dates. For this analysis, we selected the sampling dates when V_f was different from zero, because these were the dates when the net balance of in-stream processes had a significant influence on the downstream fate of nutrients.

We ran the PCA analysis with the *R* software (version 3.2.2; R project for Statistical Computing, Vienna, Austria). The rest of statistical analyses were performed with the *SPSS 11.0* statistical package. We considered statistical results to be significant at a p -value < 0.05

Results

Physical and chemical stream characterization and seasonal variation

Most of the physical and chemical variables in the receiving stream varied among seasons (Table 1). Average stream water temperature was relatively warm, with lowest values in winter and highest in summer (Table 1). Stream Q varied up to 3 orders of magnitude among seasons, being the lowest in summer and the highest in spring (Table 1). Similarly, the DF ranged from 0–100%, with higher values in winter and spring than in summer and autumn, when Q upstream of the WWTP was low or even nil. Under these seasonal conditions, average DF values were $\leq 40\%$. Water EC was moderate and varied seasonally following the inverse pattern of Q (Table 1). Regarding to nutrient concentrations, NO_3^- and SRP were consistently high, but significantly differed among seasons (Table 1). Concentration of SRP was the highest in summer and the lowest in winter. Concentration of NO_3^- was lower in spring compared to the rest of the year (Table 1). In contrast, concentrations of NH_4^+ and NO_2^- were relatively high and did not show any significant differences among seasons (Table 1). Similarly, concentration of DIN did not show any significant seasonal difference (Table 1). The $\text{NO}_3^-:\text{NH}_4^+$ molar ratio varied widely and showed that, on some dates, NH_4^+ concentration was higher than NO_3^- concentration (ratios < 1) (Table 1). Finally, the DIN:SRP molar ratio significantly differed among seasons with remarkably low values (i.e., < 16) in summer (Table 1).

The two first components of the PCA, considering the physical and chemical variables measured in the study reach, accounted for 66.8% of the variance over sampling dates (Fig. 1). The first component of the PCA (PC1) explained 47.6% of the total variance, with a positive loading of EC, temperature and NH_4^+ concentration, and a negative loading of the DF and Q (Fig. 1). Thus, this axis indicated a gradient of the relative physical and chemical influence of the WWTP effluent inputs over the temporal changes in stream hydrology (i.e., its dilution capacity). The second component of the PCA (PC2) explained 19.2% of the total variance, with a positive loading of NO_3^- concentration and the $\text{NO}_3^-:\text{NH}_4^+$ ratio (Fig. 1). Given that DIN in the WWTP effluent is relatively enriched in NH_4^+ compared to upstream (Bernal et al. 2020), this axis can be related to the influence of upstream vs. point-source inputs onto stream water chemistry.

Finally, a Kruskal-Wallis test indicated that there were significant differences among seasons for the score values of the PCA components. In relation to PC1 scores, summer cases showed significantly higher values than those in spring and winter (Kruskal-Wallis test, p -value < 0.05). In relation to PC2 scores, there were significant differences between autumn and spring (Kruskal-Wallis test, p -value < 0.05).

Temporal variation of stream net nutrient uptake

Both the magnitude and the temporal variation of net uptake rates differed among nutrients (Fig. 2 and Table 2). We found that NH_4^+ concentration significantly decreased along the reach (i.e., net uptake) on most sampling dates (i.e., 72% of all cases); yet, this proportion slightly varied among seasons (Fig. 2A). In particular, the seasons with the highest and lowest proportion of dates with net uptake were summer (85%) and spring (56%), respectively (Fig. 2A). In this sense, average V_f for NH_4^+ was consistently > 0 in all seasons (Table 2). By contrast, longitudinal profiles of NO_2^- and NO_3^- concentrations significantly increased along the reach (i.e., net release) or did not show any significant longitudinal trends (i.e. uptake = release) (Figs. 2B and 2C). Nevertheless, there were some seasonal differences between NO_2^- and NO_3^- . For NO_2^- , concentration increased along the reach in $> 50\%$ of dates, except in summer, when concentration longitudinally decreased on 50% of dates (Fig. 2B). For NO_3^- , the highest proportion of dates showing longitudinal increases in concentration was observed in autumn ($> 70\%$ of dates; Fig. 2C). In this sense, averages V_f for NO_2^- and NO_3^- were consistently < 0 in most seasons (Table 2). Further, there was a strong negative relationship between V_f for NH_4^+ and V_f for NO_3^- (linear regression, $R^2 = 0.54$, $df = 22$, $p < 0.001$, Fig. 3). There was a similar trend between V_f for NH_4^+ and V_f for NO_2^- , though this relationship was not statistically significant because the number of cases was small ($n = 5$). Finally, concentrations of both DIN and SRP showed no significant longitudinal trends (i.e. uptake = release) in 75% of the sampling dates with available data (total dates for DIN and SRP were $n = 46$ and 35 , respectively). On the few sampling dates when we observed significant longitudinal trends in concentration, average V_f for DIN was < 0 in summer and autumn and > 0 in spring (Table 2). For SRP, average V_f was > 0 in all seasons except in spring (Table 2). Nevertheless, all nutrients showed no statistically significant differences in V_f among seasons (in all cases, Kruskal-Wallis test, p -value > 0.05).

Table 2

Net nutrient uptake velocity (V_f) measured in the study stream, which receives inputs from a wastewater treatment plant effluent, on different seasons over the study period. Values of V_f were estimated from profiles of ambient nutrient concentrations that showed significant trends along the stream reach. For each nutrient, we report the range of V_f values (minimum and maximum) and the total number of data available (n), which in all cases is a fraction of all sampling dates (Table 1). We also show the average $\pm$ Standard Error values of V_f for each nutrient considering data from each season separately. For each season, n indicates the number of available data. Nutrients are: ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), dissolved inorganic nitrogen (DIN) and soluble phosphorus (SRP)

All seasons				Winter		Spring		Summer		Autumn	
V_f (mm/min)	n	Minimum	Maximum	n	Average $\pm$ SE	n	Average $\pm$ SE	n	Average $\pm$ SE	n	Average $\pm$ SE
$\text{NH}_4^+\text{-N}$	33	-0,1	11,9	5	1,4 $\pm$ 0,4	9	3,7 $\pm$ 0,7	11	1,3 $\pm$ 0,2	8	1,2 $\pm$ 0,3
$\text{NO}_2^-\text{-N}$	15	-27,7	0,5	2	-14,5 $\pm$ 5,8	6	-3,4 $\pm$ 0,8	3	0,3 $\pm$ 0,1	4	-4,7 $\pm$ 1,3
$\text{NO}_3^-\text{-N}$	26	-4,7	3,1	3	-0,6 $\pm$ 0,2	9	-1,5 $\pm$ 0,3	6	-0,6 $\pm$ 0,2	8	-0,8 $\pm$ 0,2
DIN	12	-2,4	5,2	0	N.A	6	0,2 $\pm$ 0,1	3	-0,2 $\pm$ 0,1	3	-0,7 $\pm$ 0,2
SRP	9	-1,4	2,4	2	1,4 $\pm$ 0,6	2	-0,7 $\pm$ 0,3	3	0,001 $\pm$ 0,001	2	0,1 $\pm$ 0,1

There was a negative relationship between V_f for NH_4^+ and the scores of PC1 and a positive relationship between V_f for NO_2^- and the scores of PC1 (Fig. 4). The remaining nutrients showed no relationship between V_f and the scores from PC1. For any of the studied nutrients, V_f showed no relationship with the scores of PC2

Discussion

The contribution of the WWTP effluent to the flow of the receiving stream was highly variable (from < 1 to 100%) depending on the period of the year. This pattern is typically observed in many other intermittent streams across arid and semiarid regions (Arnon et al. 2015; Martí et al. 2010; Bicknell et al. 2020), because their hydrologic regime is characterized by extreme events (i.e., floods and droughts). This finding explains the strong temporal shifts in the impact that WWTP effluent inputs have on the receiving streams of these regions as shown in our study. During high flow conditions in winter and spring, landscape features and upstream conditions have a major influence on stream physical and chemical characteristics, whereas these characteristics become increasingly subjected to WWTP effluent inputs as the dilution capacity of the receiving stream decreases during summer low flow (Keller et al. 2014). In the study stream, the impact of the WWTP effluent was dramatic for both physical and chemical variables, especially during summer and autumn when the dilution factor was well below the 40% threshold. In particular, the stream commonly dried out upstream of the WWTP input for some weeks in summer; and thus, the dilution capacity of the stream was nil. Under these conditions, water temperature and electrical conductivity were higher and oxygen concentration was relatively lower than on dates when the stream dilution factor was > 40%. Low dissolved oxygen concentration in the receiving stream could be explained by the increases in ecosystem respiration that are usually observed downstream of WWTP inputs (Gücker et al. 2006; Bernal et al. 2020). In addition, mean SRP concentration increased between 4- and 8-fold during summer, and there was a shift towards N limitation as indicated by the low DIN:SRP molar ratios. These physicochemical changes suggest that in-stream net nutrient uptake could also show marked seasonal patterns because temperature, oxygen availability, and nutrient availability can strongly influence the metabolic activity, the demand of nutrients and the preferential biogeochemical pathways of microbial in-stream communities (Butturini and Sabater 1998; Dodds et al. 2002; von Schiller et al. 2008; Ribot et al. 2017). Moreover, the intermittent hydrological regime implies that, during low flow conditions, in-stream biogeochemical processing in the receiving stream may become essential to regulate nutrient concentrations in the water column since dilution from either upstream or groundwater sources is almost negligible (Bernal et al. 2020).

The longitudinal profiles in nutrient concentrations allowed examining the temporal variability in the N and P uptake and transformation capacity of receiving stream while allowing the evaluation of the influence of the WWTP contribution to the temporal dynamics. Results indicate higher in-stream bioreactivity for DIN forms than for SRP because longitudinal profiles exhibited more significant trends (i.e., indicative of net uptake or release) for the former element than for the latter. This finding is relatively common among WWTP-receiving streams (Martí et al. 2010). Concentration of SRP did not show any significant trend along the stream on most of the sampling dates, which suggests that uptake and release processes are counterbalanced. Only in very few dates we found net uptake of SRP, although the values of V_f for SRP were in general lower than those for DIN. Low or nil net uptake of SRP could be explained by the excess of SRP from the WWTP input that could saturate the demand by microbial assemblages as well as the buffering capacity of streambed sediments (House and Denison 1998; Haggard et al. 2005).

In contrast, despite DIN concentration did not show any significant longitudinal trends on most sampling dates, each particular DIN form (NH_4^+ , NO_2^- and NO_3^-) did. Only on very few days we found a significant net uptake of DIN, but these dates did not follow any seasonal pattern. This finding suggests that the receiving stream acts more as a transformer than as a sink of N. Our results showed significant longitudinal decreases in the concentration of NH_4^+ in the study reach, an indication that this nutrient was highly processed along the stream and that uptake processes

prevailed over release processes. Regardless of the season, declines in NH_4^+ concentration were accompanied by increases in NO_2^- and NO_3^- concentrations; supporting the idea that nitrification is a prevailing process in the receiving stream over time (Merseburger et al. 2005; Bernal et al. 2020). This pattern is consistent with previous results showing that nitrification can represent up to 90% of the uptake of NH_4^+ in the study stream (Bernal et al., 2017). Similar patterns have also been described in urban streams worldwide (Cébron et al. 2003; Marti et al. 2004; Gammons et al. 2011), suggesting that high in-stream nitrification rates might be a common phenomenon downstream of WWTP effluent inputs in urban streams. These high nitrification rates can be explained by the high inputs of both NH_4^+ and nitrifying bacteria from active sludge discharged into the receiving streams from the effluents of the WWTPs (Merbt et al. 2015). We also found evidence for the occurrence of other biogeochemical processes associated with N cycling within the stream. The slope between V_f for NO_3^- and V_f for NH_4^+ was clearly below 1 (Fig. 3), indicating that the biogeochemical demand of NH_4^+ uptake was higher than expected solely from nitrification. This result suggests that assimilatory NH_4^+ uptake by photoautotrophs and heterotrophs additionally contribute to the observed declines in NH_4^+ concentration downstream of the WWTP input. In contrast, net uptake of NO_3^- occurred on few dates mostly during spring. This suggests, that except for these dates, denitrification and assimilation of NO_3^- were overwhelmed NH_4^+ nitrification. The fact that uptake efficiency for NO_3^- tends to be lower than that for NH_4^+ could explain this finding (Ribot et al. 2017). Moreover, some studies have indicated that denitrification can be limited by availability of dissolved organic matter in receiving streams (Ribot et al. 2019).

While our results strongly support the idea of the receiving stream as a nitrification hot spot, we found that the magnitude of V_f for NH_4^+ varied widely over time. Yet, there were no statistical differences in V_f for NH_4^+ among seasons, suggesting that those environmental factors clearly fluctuating with season such as temperature or light availability were likely not strongly determining the temporal variability of NH_4^+ demand by biota. Yet, we found a strong and negative relationship between V_f for NH_4^+ and the PC1 scores suggesting that the temporal variability of in-stream net uptake of NH_4^+ was closely related to changes in hydrological conditions which determine the relative influence of the WWTP effluent to stream discharge and water chemistry. Nevertheless, in contrast to the idea that stream nutrient uptake efficiency increases with decreasing stream discharge and increasing water residence time (e.g. Peterson et al. 2001; Drummond et al. 2016), we found that the magnitude of V_f for NH_4^+ was higher during relatively high flow conditions (Fig. 4). This result could be explained by the large impact of the WWTP effluent on stream physicochemistry during low flows, which lead to decreased dissolved oxygen concentrations that likely inhibited the activity of nitrifiers, despite of increases in stream water temperature. In this sense, previous studies have shown that bacterial assemblages and associated microbial activity can experience dramatic shifts when dilution factors in WWTP impacted streams are < 50% (Romero et al. 2019). Moreover, high nutrient concentrations likely had a saturation effect on in-stream NH_4^+ demand during these low flow periods when the receiving stream had a small dilution capacity. A similar saturation effect has been reported for NO_3^- uptake in urban arid land streams, despite these streams tends to be strongly N limited under pristine conditions (Grimm et al. 2005). In this study, results from the relationship between V_f for NH_4^+ and PC1 supports the saturation idea, because V_f tends to decrease in these sampling case when the dilution factor is low and the effect of WWTP input on increases in NH_4^+ concentration is high. The concomitant reduction in NO_2^- net releases (i.e., less negative values of V_f for NO_2^-) under these conditions further suggests a clear impact on the in-stream nitrification capacity. The characterization of NH_4^+ uptake kinetics of epilithic and episammic biofilms from this receiving stream additionally suggests that stream ambient NH_4^+ concentration can be above limitation (i.e., > than half saturation values) for NH_4^+ uptake and nitrification rates by microbial assemblages (Bernal et al. 2018). We further examined how NH_4^+ uptake demand varies with NH_4^+ concentration considering a wider set of pristine and human impacted streams, using the data set generated by Marcé et al. (2018) to verify if the saturation explanation holds. We found that variability in V_f for NH_4^+ from the study stream was relatively constrained and values were in the lower range of all the data set (Fig. 5) This comparison needs to be done with caution because the data set includes both gross and net uptake rates and there was no significant regression between V_f for NH_4^+ and NH_4^+ concentration (Fig. 5). However, the funnel-type pattern observed suggests that with increasing ambient NH_4^+ concentration, the biogeochemical reactivity of stream ecosystems, at least for NH_4^+ , is seriously threatened, even under hydrological conditions favoring the interaction between nutrients and biota.

The hydrologic regime and the biogeochemical reactivity are essential factors to understand the variability of nutrient uptake in streams and understand the role of these ecosystems in the regulation of nutrient cycling and exports along the river networks (Battin et al. 2008; Acuña et al. 2019). Our results are concordant with the idea that biogeochemical reactivity can be equal or even more important than hydrological opportunity to drive nutrient cycling in stream ecosystems (Marcé et al. 2018), especially in highly perturbed streams receiving chronic nutrient inputs. In particular, this study shows the relative importance of bioreactive controls (i.e., nutrient saturation) over hydrologic controls (i.e., high water residence time) on the temporal variation of nutrient uptake in WWTP-receiving streams. Moreover, the study suggests that this is influenced by the interplay between the hydrologic regime and the WWTP influence (i.e the dilution capacity), especially in Mediterranean regions. Overall, this study contributes to emphasize the distinct biogeochemical heartbeat of streams under human pressure (Grimm et al. 2005). Therefore, a better understanding of the temporal variability in nutrient uptake capacity of the receiving streams, and especially of the biogeochemical processes prevailing during low flow conditions, is important for improving the management of urban streams impacted by point-sources. In this context, our study conveys with the perspective of current studies suggesting management strategies for WWTP-receiving streams (i.e., Bicknell et al. 2020). In

particular, our study suggests that it is critical to design WWTP operation procedures taking into account both the dilution and the bioreactive capacity of receiving streams for an integrated management of water resources and their quality in urban landscapes, especially under water scarcity conditions.

Declarations

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Conflicts of interest/Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Availability of data and material

After manuscript acceptance, all data will be archived and available at Zenodo repository.

Code availability

Not applicable.

Authors' contributions

EM, FS, SB, MR participated in the design of the study. All co-authors contributed to the fieldwork on different sampling dates. SC, SB and EM conducted the compilation of the data set, the calculation of nutrient uptake metrics, and the data analyses. SC, EM, SB and MR wrote the manuscript with contributions from the rest of the co-authors.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

Not applicable.

Ethics approval

No studies involving animal or human subjects are presented in this manuscript.

Consent to participate

All authors participating in this study can withdraw their consent and stop participating at any time without prejudice.

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Figures

Figure 1

Results from the principal component analysis (PCA) based on the physical and chemical variables measure in the receiving stream over the different sampling dates. The graph shows the variability among dates (in %) explained by the two first component of the PCA. The length of the arrows indicates the relative importance of the variables with significant loading (i.e. >0.7) on both PC1 and PC2. Symbols represent the distribution of sampling dates according to the PC1 and the PC2 score space. Different symbols distinguish data by seasons, with crosses = winter; triangles = spring; squares = summer; and circles = autumn.

Figure 2

Frequency of the different trends (i.e., significant decreases or increases, and no significant changes) in the longitudinal profiles of ambient nutrient concentrations of NH_4^+ (A), NO_2^- (B) and NO_3^- (C) along the study reach of the WWTP receiving stream. The longitudinal trends, when significant, were used to estimate net nutrient uptake (estimated as uptake velocity, V_f , which could be either positive, negative or nil). Different colors in the column indicate the frequency of the three types of trends. A longitudinal decrease in ambient concentration indicates net nutrient uptake and it is labeled in black; a longitudinal increase indicates net nutrient release and it is labeled in gray; no significant trend indicates balance between nutrient uptake and release processes and it is labeled in white. For each dissolved inorganic nitrogen form, the frequency of the different longitudinal trends is reported for all data together and for data collected in each season separately.

Figure 3

Linear relationship between net uptake velocities (V_f) for NH_4^+ and for NO_3^- measured in the WWTP-receiving stream over the study period ($n = 23$ sampling dates). The equation for the relationship and the statistic results are shown. Different symbols distinguish data by seasons, with crosses = winter; triangles = spring; squares = summer; and circles = autumn.

Figure 4

Relationships between net uptake velocity (V_f) for NH_4^+ (A) and for NO_2^- (B) and the scores from the principal component 1 (PC1) of the PCA conducted with physical and chemical characteristics of the receiving stream. The arrow above the graphs indicates the significant variables

(loading >0.7) associated with the PC1 and their respective positive (+) or negative (-) weight. Different symbols distinguish data by seasons, with crosses = winter; triangles = spring; squares = summer; and circles = autumn.

Figure 5

Relationship between NH₄⁺ concentration and uptake velocity (V_f) for NH₄⁺ considering results from this study (empty circles) together with results from several other studies done in pristine and human altered streams worldwide (black circles), which were compiled in Marcé et al (2018). Detailed information on the published sources for the compiled data set is provided in the supplementary material (Appendix 1).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [APPENDIXCastelaretal.docx](#)